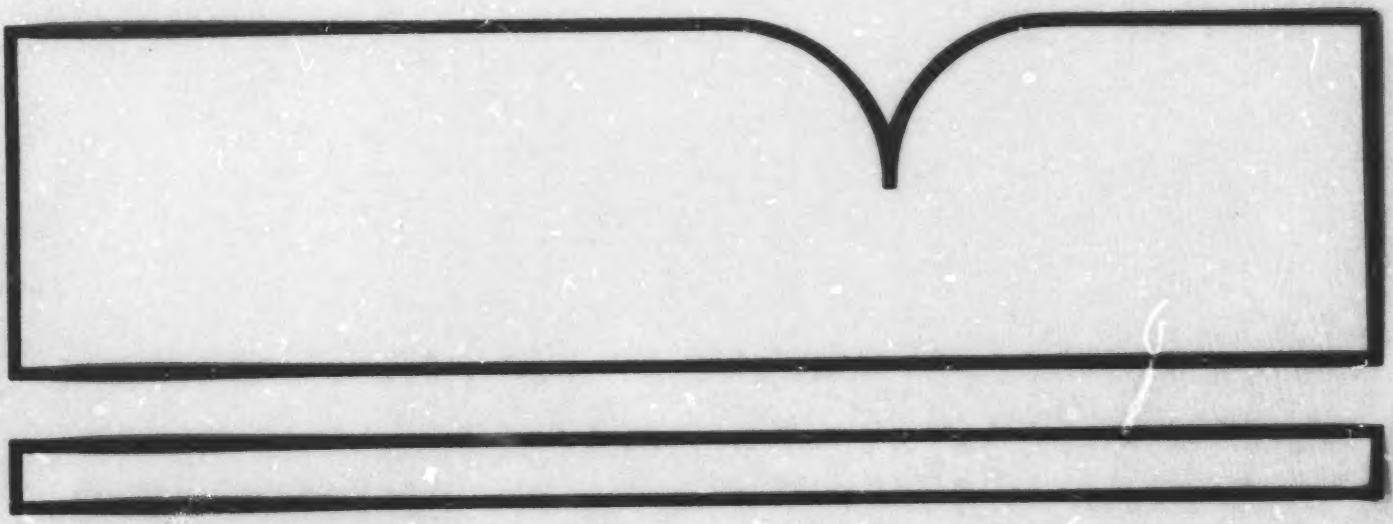
PB86-160298

Influence of Microbial Adaptation on the Fate of Organic Pollutants in Ground Water

(U.S.) Robert S. Kerr Environmental Research Lab., Ada, OK

1985



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completi

1. REPORT NO.	2.	3.
EPA/600/D-86/023		PB86-160298

4. TITLE AND SUBTITLE
INFLUENCE OF MICROBIAL ADAPTATION ON THE FATE OF ORGANIC POLLUTANTS IN GROUND WATER

5. REPORT DATE
1985

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
John T. Wilson, James F. McNabb and Jack W. Cochran[1]
T. Helen Wang, Mason B. Tomson and Philip B. Bedient[2]

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
[1]R.S. Kerr Environmental Res. Laboratory, U.S. EPA
P.O. Box 1198
Ada, OK 74820

[2]Dept. Environmental Science & Engineering
Rice University
Houston, TX 77001

10. PROGRAM ELEMENT NO.
CBPC1A

11. CONTRACT/GRANT NO.
CR-806931

12. SPONSORING AGENCY NAME AND ADDRESS
Robert S. Kerr Environmental Research Laboratory
U.S. Environmental Protection Agency
Post Office Box 1198
Ada, Oklahoma 74820

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/15

15. SUPPLEMENTARY NOTES
Published in ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY, 4:721-726, 1985.

16. ABSTRACT

A plume of contaminated groundwater originating from an abandoned disposal pit for wood-creosoting waste was characterized. The important organic contaminants in the plume include naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, dibenzofuran and fluorene at individual concentrations of 1,000 to 100 µg/L. Core material from the site was studied to determine if organisms in the subsurface could adapt to this waste and if biological activity influenced the disposition of the plume. Biodegradation of these organic pollutants in subsurface material from the margin of the plume was rapid. No biodegradation of the pollutants was detected in pristine subsurface material from the same geological structure. As a result of this adaptation, the disposition of the plume was not controlled by the rate of utilization of the pollutants by the microorganisms but by the extent of utilization allowed by the supply of oxygen.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO THE PUBLIC.	UNCLASSIFIED	8
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA/600/D-86/023
1985

Environmental Toxicology and Chemistry Vol. 4, pp. 721–726, 1985
Printed in the USA. Pergamon Press Ltd.

0730-7268/85 $3.00 + .00

SYMPOSIUM
Biodegradation of Chemicals in the Subsurface Environment

INFLUENCE OF MICROBIAL ADAPTATION ON THE FATE OF ORGANIC POLLUTANTS IN GROUND WATER

JOHN T. WILSON,* JAMES F. MCNABB and JACK W. COCHRAN
U.S. Environmental Protection Agency, R.S. Kerr Environmental Research Laboratory, Ada, Oklahoma 74820

T. HELEN WANG, MASON B. TOMSON and PHILIP B. BEDIENT
Department of Environmental Science and Engineering, Rice University, Houston, Texas 77001

(*Received* 26 *July* 1984; *Accepted* 1 *October* 1984)

Abstract—A plume of contaminated groundwater originating from an abandoned disposal pit for wood-creosoting waste was characterized. The important organic contaminants in the plume include naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, dibenzofuran and fluorene at individual concentrations of 1,000 to 100 μg/L. Core material from the site was studied to determine if organisms in the subsurface could adapt to this waste and if biological activity influenced the disposition of the plume. Biodegradation of these organic pollutants in subsurface material from the margin of the plume was rapid. No biodegradation of the pollutants was detected in pristine subsurface material from the same geological structure. As a result of this adaptation, the disposition of the plume was not controlled by the rate of utilization of the pollutants by the microorganisms but by the extent of utilization allowed by the supply of oxygen.

Keywords—Adaptation Acclimation Groundwater Organic pollutants

INTRODUCTION

The United States is becoming acutely aware that inappropriate disposal of hazardous waste is a threat to our groundwater resources. A clear understanding of the behavior of these wastes in the subsurface environment is required to properly assess the environmental damage from existing waste disposal sites and to identify the most appropriate approaches for containment or cleanup of the waste.

At any particular site, the behavior of a waste is influenced by the hydrogeology of the site, sorption to the particular subsurface materials and biological and nonbiological transformations. The relative influence of these processes and conditions on the behavior of a waste can vary from compound to compound, between sites and, on occasion, within sites. If an important process or condition is ignored in a projection of environmental fate, either through indifference or from lack of the appropriate information, that projection can be seriously misleading.

Hydrocarbons derived from petroleum or other fossil fuels are important pollutants of groundwater. Sources include gasoline from spills or leaky storage tanks, waste from abandoned illuminating-gas plants, industrial impoundments and landfills. The behavior of these compounds is subject to all the conditions and processes mentioned above. They should provide a good test of our capacity to characterize the behavior of an organic waste in the subsurface environment.

Adaptation of subsurface microbes to components in creosote wastes has been documented pre-

Publisher's notice to authors and subscribers: The National Center for Ground Water Research determined in 1981 that *ground water* should be two words. The Editorial office will honor this convention beginning with Volume 5.

*To whom correspondence regarding biotransformations may be addressed. Correspondence regarding analytical environmental chemistry may be addressed to Dr. Tomson and that regarding hydrology and computer modeling to Dr. Bedient.

Although the research described in this article has been funded in part by the U.S. Environmental Protection Agency, it has not been subject to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

Presented at the Symposium on Biodegradation of Chemicals in the Subsurface Environment, Fourth Annual Meeting of the Society of Environmental Toxicology and Chemistry, Arlington, Virginia, November 6–9, 1983, and sponsored by Procter & Gamble Company, Cincinnati, Ohio.

viously by Ehrlich et al. [1,2]; however, activity at the site studied was under strictly anaerobic conditions and was restricted to the methanogenic degradation of phenolic compounds. There was no adaptation to or activity against naphthalene or other polynuclear aromatic compounds under anaerobic conditions. Ogawa et al. [3] reported the aerobic degradation of a number of polynuclear aromatic compounds in water from a contaminated well. There was no degradation in water from an uncontaminated well in the same aquifer, indicating that adaptation had occurred, at least in the environment of the contaminated well. The well may have allowed organisms from the surface to colonize the bore hole and the region of the formation around the well. There was no evidence that the aquifer at large was adapted to the waste.

This report is a preliminary attempt at a comprehensive assessment of the behavior of pollution from creosote waste at a site in the Gulf Coast region of Texas. It emphasizes the importance of microbial adaptation in determining the behavior of the waste.

MATERIALS AND METHODS

Analysis of water from monitoring wells

Oxygen levels were determined by flushing the bore hole with nitrogen or argon, pumping the well to produce fresh water from the formation and then lowering a YSI oxygen electrode into the well. Chloride levels were determined by the Hach Kit method for total chloride. Organic compounds were sampled by pumping 100 to 1,000 ml of well water (depending on the extent of contamination) through a C_{18} reversed phase Sep-Pak trap (Waters Associates). The compounds were eluted in 4.0 ml methylene chloride. The extract was concentrated under nitrogen and analyzed by gas chromatography using a fused-silica capillary column and flame-ionization detector.

Biotransformation studies

Authentic subsurface material was used to construct small 45-ml, static, batch microcosms as has been described previously [4,5]. Organic compounds were extracted from the subsurface material by shaking in a chloroform–methanol azeotrope for 18 h. The extract was separated from the aqueous phase and solids, dried through sodium sulfate, concentrated by Kuderna-Danish distillation and analyzed by gas chromatography using a fused-silica capillary column and a flame-ionization detector.

Computer modeling

Simulations were done using a one-dimensional analytical solution to the universal solute transport equation. Sorption was assumed to be linear; degradation was assumed to be first-order with respect to concentration.

RESULTS

Geological setting and history of the site

The abandoned pits that are the source of pollution received wastes from a wood-creosoting process from 1952 to 1975. They have now been filled and the land developed for other purposes.

The waste pits and plume of contamination are contained in unconsolidated material deposited by an egressing river delta. From the present surface to a depth of 5 to 6 m is a complex pattern of interlayered sands and clays that probably were deposited as meander belts by a stream. From 5 to 6 m down to 8 to 9 m is a layer of poorly sorted sand that was probably deposited in a near-shore environment. Below 8 to 9 m is a layer of clay that was probably deposited in an off-shore environment.

The lower layer of clay seems to be uniformly distributed across the entire site; its transmissivity is low, 10^{-9} to 10^{-10} cm/s. The sand is somewhat variable over the site; its transmissivity near the waste pit is much higher than that of the clay, 10^{-3} to 10^{-4} cm/s. The present water table is 7.6 to 8.5 m deep. A plume of contaminated water is moving laterally through the sand away from the disposal pit. All of the monitoring wells are screened in the relatively shallow zone of water-saturated sand situated above the clay. The present flow velocity in the sand is estimated to be 10 m/year.

Characterization of the plume

When the concentrations of organic pollutants, oxygen and electrolytes in the monitoring wells are compared, the wells fall into four categories (Table 1, Fig. 1). Wells 3 and 14 were constructed at locations 85 and 350 m from the pits in areas that are removed from the hydrological influence of the waste pit. These areas are pristine; none of the organic pollutants were detected in these wells, oxygen was present and chlorides and conductivity were low.

Wells 30, 9, 2 and 27 penetrated the plume of contamination. The concentration of the organic pollutants was high and the concentration of indi-

Table 1. Distribution of organic pollutants, oxygen and electrolytes in monitoring wells at the Conroe, Texas, creosote-waste site

	Location of monitoring wells[a]											
	Pristine zone		Plume of contamination				Zone of Active treatment				Treated zone	
Parameter	3	4	30	9	2	27	28	29	8	5J	26	5A
Cl^- (mg/L)	8	16	22	21	50	29	47	40	49	28	34	48
Conductivity (μmhos/cm)	88	110	112	125	282	139	250	240	252	230	220	241
O_2 (mg/L)	1.4	No data	0.1	0.2	<0.1	0.3	0.3	<0.1	0.2	1.6	1.7	0.3
Naphthalene (μg/L)	<0.1	<0.1	1,200	1,600	650	3,500	49	67	90	65	1.4	14
1-Methylnaphthalene (μg/L)	<0.1	<0.1	470	370	220	610	3.5	8.8	15	22	2.2	1.5
2-Methylnaphthalene (μg/L)	<0.1	<0.1	650	710	390	1,300	5.7	12	26	37	2.1	2.5
Dibenzofuran (μg/L)	<0.1	<0.1	420	250	140	290	2.3	3.7	11	20	5.1	<0.1
Fluorene (μg/L)	<0.1	<0.1	300	230	120	290	2.0	2.8	13	11	7.9	1.1

[a]See Fig. 1.

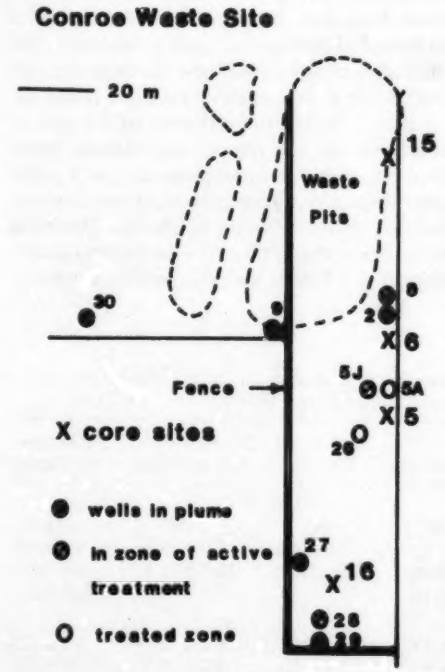


Fig. 1. Location of monitoring wells and sampling sites for core material at a disposal site for wood-creosote waste at Conroe, Texas.

vidual compounds remarkably uniform. Compared to the pristine water, oxygen was greatly depleted and the concentration of chloride and the conductivity of the water was higher, probably reflecting salts that were in the creosote wastes.

Wells 28, 29 and 8 were in a zone of active treatment. Oxygen concentrations were depressed and chloride and conductivity were elevated, indicating the arrival of the plume. However, the concentration of each organic pollutant was reduced roughly one order of magnitude. Wells 26 and 5A were in a treated zone. The concentrations of the organic pollutants in them were much reduced and, in the case of well 26, the concentration of oxygen was close to that expected for pristine conditions. These wells had elevated concentrations of chloride and higher conductivity than pristine water, indicating that they sampled renovated water from the plume.

Well 5J was anomalous; it produced water containing appreciable concentrations of the organic pollutants as well as high concentrations of oxygen. The simplest explanation is that this well straddled the zone of active treatment, either vertically or horizontally, and produced oxygenated water from one region and water polluted with organics from another.

The locations of the monitoring wells are shown in Figure 1. The direction of groundwater flow is

down and to the left. Wells 30, 9 and 2 intercept the plume as it leaves the waste pit. The water probably has undergone little renovation due to sorption or degradation of the organics. Well 27 shows that the plume extends at least 60 m downgradient without appreciable renovation.

The wells in the zone of active treatment and in the treated zone are scattered along the margin of the plume. In several cases, wells with intense pollution are very close to wells receiving considerable treatment. (Compare wells 2 and 8 or 27 and 28.) This indicates that the zone of treatment can be very narrow, perhaps as little as 10 m in horizontal extent.

Biological activity in subsurface material

The water-saturated sands from two sites within the zone of treatment (sites 5 and 16) and from a pristine site (site 14) were examined for biological activity against the organic pollutants. The samples were acquired in a manner that precluded contamination by surface microorganisms [4]. Oxygen was added to the pore water of the subsurface material to a final concentration of at least 1.0 mg/L. Because nonautoclaved material from site 16 contained appreciable quantities of some of the organic contaminants it was not further amended. Material from wells 5 and 14 and autoclaved material from site 16 were essentially free of organic pollutants, so they were amended with the organic compounds listed in Table 1 to a final concentration of 20 to 120 ng/g dry subsurface material. Because acenaphthene was present in the nonautoclaved material from site 16, it was also added to the other subsurface material.

There was no detectable biological activity in the material from the pristine site (Table 2). In fact, the disappearance of the compounds was somewhat more rapid in the autoclaved material. However, there was rapid biotransformation of the organic compounds in the material from the zone of treatment. Material from site 5 was particularly active against any of the organic compounds.

Material from site 6 was examined for activity against a series of alkyl benzenes. The results will be presented elsewhere; however, biodegradation occurred in material from site 6 but was not detected in material from a pristine site. Additionally, material from sites 6 and 15 is presently being characterized by participants in the National Center for Ground Water Research to further elucidate the nonbiological interactions between the organic pollutants and the subsurface material.

Site-specific interpretation of rates of degradation

The specific concentration of any one of the organic pollutants in the plume is simultaneously controlled by several processes, including dilution due to dispersion, sorption to subsurface solids and biological and nonbiological degradation. The contribution of any one of these processes can only be evaluated in the context of the other processes.

Figure 2 depicts the influence of the rate of degradation on the fate of naphthalene under conditions that are appropriate to the Conroe creosote-waste site. Transport of naphthalene was simulated for the following conditions: The initial concentration was 1,000 μg/L; the dispersion coefficient D was 100 m; the flow velocity was 10 m/

Table 2. Biological activity against organic pollutants inferred from the decrease in the quantity (% per week) of compound extracted from subsurface material from Conroe, Texas, after incubation at 25°C

Sampling site	Naphthalene	2-Methyl-naphthalene	Dibenzofuran	Fluorene	Acenaphthene	1-Methyl-naphthalene
Contaminated site						
5	>160[a]	>160[a]	>180[a]	>92[a]	>130	>160[a]
5 (autoclaved)	8.2 ± 6.3[b]	6.5 ± 4.6	4.9 ± 3.6	3.4 ± 4.0	5.0 ± 3.1	5.6 ± 4.6
16	>257[a]	8.0 ± 2.5	No data	No data	32.5 ± 11.8	No data
16 (autoclaved)	7.7 ± 2.7	6.1 ± 2.9	4.9 ± 3.3	11.1 ± 6.9	7.9 ± 3.2	5.9 ± 3.2
Pristine site						
14	3.7 ± 4.0	3.5 ± 2.9	3.9 ± 3.6	6.4 ± 4.7	6.6 ± 2.1	3.6 ± 3.0
14 (autoclaved)	11.3 ± 6.2	11.1 ± 5.7	9.9 ± 6.2	9.4 ± 6.5	9.2 ± 6.9	10.9 ± 5.2

The material was acquired from the sites in June 1983; triplicate samples were analyzed after 0, 1, 2, 4, and 8 weeks of incubation.

[a]Below detection limit (1 ng/g) before first sampling interval.

[b]95% confidence interval ($s_b t_{0.05}$) on the zero-order rate of decay.

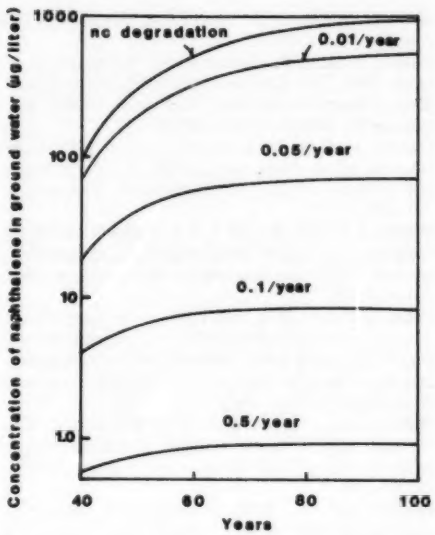


Fig. 2. A computer simulation of the effect of the rate of degradation on the concentration of naphthalene that can reach an arbitrary boundary 200 m from the waste disposal pits.

year; and the retardation due to sorption was 3, as determined by the behavior of naphthalene in core material in the laboratory. A radius of 200 m was selected as an arbitrary boundary between a site scale and a regional scale of concern. Figure 2 projects the influence of degradation on the concentration of naphthalene that can be expected to reach that boundary over the next 70 years. A rate of degradation as low as 5% per year should have a substantial influence on the breakthrough of naphthalene, whereas a rate of 50% per year should depress the breakthrough of naphthalene by three orders of magnitude. A rate of 50% per year is close to the detection limit of the biodegradation assay, which is approximately 2% per week. Rates of degradation in subsurface material from the zone of treatment were one to two orders of magnitude greater than 50% per year and could easily account for the extensive renovation of groundwater in the zone of treatment over the relatively short distances between monitoring wells.

DISCUSSION

Occurrence of adaptation

Adaptation is a phenomenon that can be brought about by several mechanisms or processes, including growth of active organisms, enzyme induction and mutation or other genetic changes. The term simply refers to an increase in the rate of biotransformation of a substance resulting from exposure to that substance [6]. Clearly, microbes in the water-saturated sand at the Conroe creosote-waste site adapted to the six organic pollutants considered in this study. This adaptation results in rates of biotransformation of at least one to two orders of magnitude greater than could be detected in material that was not exposed to the pollutants. This result is similar to experience with surface sediments. Herbes and Schwall [7] found that the rate of degradation of naphthalene in oil-contaminated stream sediment was 2,350% per week, while the rate of degradation in uncontaminated sediments was less than 7% per week. Additionally, Herbes [8] found that the rate of naphthalene degradation in stream sediments downstream of a coal-coking plant was 1,300% per week.

Significance of adaptation

Once adaptation occurs, biotransformation is so rapid that it can be considered instantaneous within the context of slow groundwater flow. As a result, the quantitative prediction of the effect of biological activity or the fate of a pollutant shifts from a consideration of the kinetics of utilization of the substrate by the microorganisms to consideration of the extent of utilization allowed by geochemical constraints on metabolism and the rate of supply to the subsurface environment of that limiting requisite for metabolism. In the case of the Conroe creosote-waste site, the disposition of the plume is controlled by the supply of oxygen to its margins, either by diffusion from the unsaturated zone or by admixture of oxygenated groundwater through dispersion.

As a result, predicting the behavior of a contamination plume is greatly simplified—there is no need to characterize the biological activity. However, there is need for tools to quickly and inexpensively recognize whether adaptation has occurred in a particular subsurface environment. Also needed is a delineation of the conditions for which adaptation can be expected in the subsurface and methods to predict the time required for adaptation.

Significance of the absence of adaptation

If adaptation fails to occur, predictions of the influence of biological activity and the fate of a pollutant will be difficult. As demonstrated in Figure 1, rates of biotransformation that are much

below the present detection limit can have considerable environmental significance. Work is under way in the National Center for Ground Water Research to lower the detection limit through the use of radiolabeled substrates.

CONCLUSIONS

Microbes in the subsurface environment adapted to degrade a series of polynuclear aromatic hydrocarbons in a plume of pollution originating from wood-creosoting waste. Following adaptation, the supply of oxygen controlled the disposition of the plume. Slow rates of degradation in unadapted material can have profound effects on the fate of compounds in the subsurface environment on a regional scale.

Acknowledgement—The authors are participants in the National Center for Ground Water Research. The research was funded in part by the U.S. Environmental Protection Agency through cooperative agreement No. CR806931-02 to the National Center for Ground Water Research.

REFERENCES

1. **Ehrlich, G.G., E.M. Godsey, D.F. Goerlitz** and **M.F. Hult.** 1983. Microbial ecology of a creosote-contaminated aquifer at St. Louis Park, Minnesota. *Dev. Ind. Microbiol.* **24**:235–245.
2. **Ehrlich, G.G., D.F. Goerlitz, E.M. Godsey** and **M.F. Hult.** 1982. Degradation of phenolic compounds in ground water by anaerobic bacteria: St. Louis Park, Minnesota. *Ground Water* **20**:703–710.
3. **Ogawa, I., G.A. Junk** and **H.J. Svec.** 1981. Degradation of aromatic compounds in groundwater, and methods of sample preservation. *Talanta* **28**: 725–729.
4. **Wilson, J.T., J.F. McNabb, D.L. Balkwill** and **W.C. Ghiorse.** 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. *Ground Water* **21**:134–141.
5. **Spain, J.C.** and **D.A. VanVeld.** 1983. Adaption of natural microbial communities to degradation of xenobiotic compounds: Effects of concentration, exposure time, inoculation, and chemical structure. *Appl. Environ. Microbiol.* **45**:428–435.
6. **Wilson, J.T., J.F. McNabb, B.H. Wilson** and **M.J. Noonan.** 1983. Biotransformation of selected organic pollutants in ground water. *Dev. Ind. Microbiol.* **24**:225–233.
7. **Herbes, S.E.** and **L.R. Schwall.** 1978. Microbial transformation of polycyclic aromatic hydrocarbons in pristine and petroleum-contaminated sediments. *Appl. Environ. Microbiol.* **35**:306–316.
8. **Herbes, S.E.** 1981. Rates of microbial transformation of polycyclic aromatic hydrocarbons in water and sediments in the vicinity of a coal-coking wastewater discharge. *Appl. Environ. Microbiol.* **41**:20–28.